SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY, NANDED.

Synopsis of Proposed Research Plan

"Screening and Evaluation of Certain Wild Vegetables Belonging to Family Cucurbitaceae for their Biological Activities."

Submitted for the degree of

Doctor of Philosophy in Botany

Under

Faculty of Science and Technology

Submitted

By

Mr. Sachin Sarjerao Shinde
M.Sc. Botany (CSIR NET-JRF AIR-96, MH-SET)
Research Scholar

Under the Guidance
of

Dr. B D. Gachande
Professor and Head
Department of Botany, Microbiology, and Biotechnology
N.E.S. Science College, Nanded.

[Re-accredited with "A" Grade by NAAC (CGPA-3.38)]
DST, UGC CPE Status

March, 2022

Research Title:

"Screening and Evaluation of Certain Wild Vegetables Belonging to Family Cucurbitaceae for their Biological Activities."

1. Introduction:

Wild herbs have been used worldwide for decades as part of the human diet. Edible herbal medicines that have a therapeutic value may go a long way in getting drugs from these wild vegetables whose plant parts can be easily harvested continuously. Therefore, there is a need to evaluate edible wild herbs for therapeutic use (Maroyi et al., 2011) that may benefit the community. The current study aims to test and evaluate selected wild herbs as a food source for traditional healers in the Nanded region for their antimicrobial and anticancer activities. The use of traditional medicine is not new as it seemed to be incredibly popular in the 1990s (FAO, 1997). Other studies in Ethiopia have shown that many tribal months have been given extensive information about the use of wild vegetable resources, this is especially true regarding the use of medicinal plants (D. Abebe and A. Anteneh., 1993). Several phytochemicals present in wild herbs are promising options for improving the effectiveness of cancer treatment. Most of these phytochemicals are naturally occurring biologically active compounds with excellent anticancer properties. The development of effective therapeutic effects and adverse effects of phytochemical-based antimicrobial and anticancer therapy begins with the testing of natural extracts (from dried plant extracts) to detect potential antimicrobial and anticancer activity. The nutrients found in plants known as phytochemicals can act as important resources in new medicines.

Wild herbs are collected in nature and widely used around the world, but an understanding of therapeutic properties, safety, and effectiveness has become a major problem in recent years (Sekeroglu and Koca, 2010). The increase in the prevalence of common pathogenic microorganisms that are resistant to viruses over the years requires new antimicrobial agents. Wild herbs provide new antimicrobial agents and a large number of new drug components (Nascimento et al., 2000; Obeidat et al., 2012). Wild and harvested vegetables have fewer side effects compared to synthetic or semi-synthetic drug agents. A large number of early studies have been conducted to identify potent antimicrobial agents (Obeidat et al., 2012; Molla et al., 2010).

Cancer is a major public health problem worldwide. According to the World Health Organization (WHO) in 2019, cancer is the first or second leading cause of death before the age of 70 in 112 countries in 183 countries and is ranked third or fourth in 23 countries. Globally, an estimated 19.3 million new cancer cases and nearly 10.0 million cancer deaths by 2020 (Hyuna Sung, 2020). By 2020, the diagnosis and treatment of cancer were interrupted by a coronavirus (COVID-19) epidemic that led to delays in diagnosis and treatment that could lead to a temporary decrease in cancer cases followed by an increase in the prevalence and eventually increased mortality (Rebecca L. et al., 2021). The global cancer burden is expected to be 28.4 million cases by 2040 due to statistical changes, although this may be due to an increase in risk factors related to global trade and the growing economy (Hyuna Sung. Et al., 2020). The burden of cancer and mortality is growing rapidly around the world as a result of changes in the way people live or in rural areas, cancer becomes an epidemic (Susan J. Curry, et al., 2003).

2.Review of literature and development in the subject (Previous work done in a relevant area):

M.G. Mokganya, M.P. Tshisikhawe, (2019) writes about thirteen wild vegetables that are reportedly used by Vhavenda in the Vhembe District Municipality, South Africa. He concluded that eating enough spicy vegetables for members of the Cucurbitaceae family in the Vhembe district was seen as improving their health by lowering their diabetes and high blood pressure. Rajasree et al. (2016) reported that cucurbit plants are a rich source of protein that puts many of the nutrients needed for good health. As an important support for these findings, Rahman and Sousa-Poza (2010) state that when we eat vegetables, we get the nutrients we need to live a healthier life.

The use of wild herbs for medicinal purposes was also documented in Zimbabwe (Maroyi, 2011). He wrote about 67 species of 45 edible wild plants and 30 families used in the Nhema community area, focusing on edible portions and their role in meeting daily food needs and food shortages.

Most of the studies conducted in the Vhembe district in the Limpopo Province of South Africa focused on recording and testing edible wild vegetables (Nesamvuni et al., 2001; Maanda and Bhat, 2010).

In some parts of the world such as the USA, Europe, and Australia, the role of healthy wild vegetables in conservation is also recognized as functional, nutritious foods, or phytonutrients (Craig, 1999). He suggested that several herbs help lower blood cholesterol levels, provide some protection against cancer, and stimulate the immune system. In addition, foods, where culinary herbs are used sparingly in the diet, provide a variety of active phytochemicals that promote health and protect against chronic diseases.

In addition, Chen (2000) emphasized that the concept of diet as a medicine has been present in Chinese medicine since ancient times. Falconer and Arnold (1991) emphasize the fact that many wild plants have many functions, for example, providing nutrients, promoting a variety of foods, feeding livestock, and giving people medicine.

In India, edible wild vegetables have been identified as an important host when it comes to the social and economic well-being of communities. In 2019, Hanumantappa Bherigi Nayaka Fifteen wild herbs were reported to have medicinal value consumed by the Hyderabad-Karnataka region in the Karnataka region of India. He reported that wild herbs are used to treat high blood pressure and diabetes.

3. Objectives of Research/ Proposed Hypothesis:

1. Collection, identification, and validation of certain selected wild vegetables of Cucurbitaceae.
2. Evaluation of certain wild vegetables in terms of their chemical composition.
3. Screening of phytochemicals.
4. Screening of antioxidant potential and antimicrobial assays.
5. Characterization of phytochemicals by HP-TLC/GC-MS/HR-LCMS or any other selected technique.
6. An anticancer activity test using the SRB/MTT assay.

4. Methodology to be adopted:

Phase-I: Literature survey, collection/authentification/identification of certain wild vegetables:

Our primary focus is on a literature survey to know the previous work of the research scientist in the same research area. In-depth information was collected about the wild vegetables from traditional healers of the Nanded district with extensive field visits by using all COVID-19 safety measurements. Wild vegetables are collected from the studied area and identified by using the flora of the Marathwada region (Naik V. N, 1998).

Phase-II: Preparation and Extraction of plant samples:

The collected plant parts are shade dried for two weeks afterwhich it was grounded into a fine powder using a pestle and mortar, the dried samples (plant powder) were kept in tight bottles. The Soxhlet extraction unit will be used for the ethanolic extract of plant powders (Harborne J. B., 1973).

Phase III: Phytochemical analysis extracts for quality and quantity:

Phytochemical testing will be performed on the ethanolic plant extraction using the normal process of identifying phytoconstituents as described by Trease and Evans (1989) and Kankia H.I. (2014).

Phenolic content ratio: Estimation of polyphenolic content from selected plants will be determined by previously reported methods (Bray and Thorpe, 1954). The phenolic compound reacts with the oxidizing agent phosphomolybdate present in the Folin-Ciocalteau reagent, the resulting reaction product being a complex blue color with a high dispersion of 660 nm. Briefly, the reaction mixture contains 1 ml extracted from each plant sample, 2 ml of distilled water, 0.5 ml of Folin-Ciocalteau reagent, and 2 ml of Na2CO3 (20%). The mixture will be stored in a water bath for 1 minute and after cooling, the absorption will be measured at 660 nm in the correct position using a UV-VIS spectrophotometer. The total amount of polyphenols will be calculated by adjusting the measuring curve using a different mixture of rutin (500 lg / ml) and an unknown amount of phenolics measured as mg / g of samples (R.N. Gacche, N.A. Dhole, 2011).

Flavonoid content ratio: The total flavonoid content of each plant sample is measured in the form of aluminum chloride described elsewhere (Chang et al., 2002). The reaction mixture contains 1 ml of plant sample, 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml (1 M) of potassium acetate, and 5.6 ml of distilled water. The mixture was placed for 30 minutes at room temperature and the absorption was recorded at 415 nm using a UV-VIS spectrophotometer. The standard curve was prepared using serial dilutions of quercetin (100 lg/ml). The collection of flavonoid samples for each plant was calculated using the standard curve and quercetin values (mg/g).

Phase IV: Antimicrobial activities of certain wild vegetable extracts:

Ethanol plant extracts will be tested against various bacterial and fungal microorganisms to assess the biological barrier tested using standard protocols.

Phase-V: Separation of Phytochemicals from extracts by using HPTLC/GC-MS:

The plant extracts usually contain various types of phytochemicals. Therefore, the isolation of these phytochemicals will be carried out by using separation techniques such as HPTL/GC-MS. This separation technique is carried out from an outsourcing facility available at the specific research center.

Phase-VI: Screening and Evaluation of antimicrobial and anticancer potential of selected vegetable:

The anticancer activity will be performed using a test of the yellow tetrazolium MTT (3- (4, 5-dimethyl thiazolyl-2) -2, 5-diphenyltetrazolium bromide) and made into a discharge center located in a specific study area.

Phase-VII: Thesis writing and submission:

In the final phase, scientific thesis writing will be done and submitted to the research university.

5.Importance of study/society application:

International status:

The global cancer burden uses the GLOBOCAN 2020 estimates of cancer and mortality cases developed by the International Agency for Research on Cancer. Globally, an estimated 19.3 million new cancer cases (18.1 million without nonmelanoma skin cancer) and approximately 10.0 million cancer deaths (9.9 million without nonmelanoma skin cancer) occur by 2020. an estimated 2.3 million adolescents (11.7%), followed by lung (11.4%), colorectal (10.0%), prostate (7.3%), and stomach cancer (5.6%). Lung cancer remains the leading cause of death for cancer patients, with an estimated 1.8 million (18%), followed by colorectal (9.4%), liver (8.3%), stomach (7.7%), and women's breasts (6.9%) (GLOBOCAN-2020).

Europe accounts for 22.8% of cancer cases and 19.6% of cancer deaths, although it represents 9.7% of the world's population followed by 20.9% of American cases and 14.2% of deaths worldwide.

National status:

A report by the National Cancer Registry Program 2020, released by the Indian Council of Medical Research (ICMR) and the National Center for Disease Informatics & Research (NCDIR), Bengaluru, estimates that by 2020 the incidence of cancer in the country will reach 13.9 lakhs and possibly increase. at 15.7 lakhs by 2025, based on current trends. This data is based on cancer-related information collected from 28 Population-based Cancer Registries (PBCRs) and 58 Hospital Based Cancer Registries (HBCRs) that provided cancer data.

Today, there are many different types of cancer treatments, such as surgery, chemotherapy, radiation, and so on, but they may also produce side effects. Therefore, there is a need to develop novel and effective herbal drugs as well as free side effects for cancer treatment.

6. Proposed work Plan/ Formulation and structure of the study

Sr. No.	Year	Proposed work	Tentative Duration
1.	Ist Year	Literature survey	**First Year** (Aug.21-Aug.22)
		Collection/identification and authentification of certain wild vegetables of Cucurbitaceae from the Nanded District	
		Drying and Extraction of plant samples	
		Screening of certain wild vegetables for their chemical composition	
2.	2nd Year	A qualitative test of plant extracts for the presence or absence of secondary metabolites	**Second Year** (Aug.22-Aug.23)
		Quantitative testing of selected plant extracts	
		Antimicrobial activity of plant extracts	
3.	3rd Year	Separation of Phytochemicals by using HP-TLC/GC-MS	**Third Year** (Aug.23-Aug.24)
		Screening and Evaluation of certain Plant extracts from wild vegetables for their anticancer activity by using SRB/MTT Assay	
		Thesis writing and submission	
• Presentation and publication of research work and attending conferences will be done alongside all the activities mentioned in the above table.			

References:

1. Bray, H.G., Thorpe, W.V., 1954. Analysis of phenolic compounds of interest in metabolism. Meth. Biochem. Anal. 1, 27–30.
2. Chang, C., Yang, M., Wen, H., Chern, J., 2002. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. J. Food Drug Anal. 10, 178–182.
3. Chen, J., 2000. Regulatory control of functional food in China. Scadinavian Journal of Nutrition 44, 130–131.
4. Craig, W.J., 1999. Health-promoting properties of common herbs. American Journal of Clinical Nutrition 70, S491–S499.
5. D. Abebe and A. Anteneh., 1993. Medicinal Plants and Enigmatic Health Practice ofNorthern Ethiopia, Monograph, Addis Ababa, Ethiopia.
6. Falconer, J., Arnold, J.E.M., 1991. Household food security: an analysis of socio-economic values. Community Forestry Note 1. Forest Trees and People. FAO, Rome, Italy.
7. FAO., 1997. Medicinal Plants for Forest Conservation and Health Care. Food and Agriculture Organisation of the United Nations.

8. Hanumantappa Bherigi Nayaka., 2019. Medicinal uses of selected wild edible vegetables consumed by Hyderabad-Karnataka region of Karnataka State in India, Asian Journal of Pharmacy and Pharmacology, 6(1): 70-75.
9. Harborne J. B., 1973. Phytochemical Methods. Chapman and Hall, Ltd., London, 49-188.
10. Hyuna Sung, Jacques Ferlay, Rebecca L. Siegel, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, Freddie Bray. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, Cancer J Clin. 71:209–249.
11. Kankia, H. I., 2014. Phytochemical analysis and antimicrobial activity of crude extract of Datura metal leaves, Applied Science Research, 6 (6):138-143 ISSN 0975-508X.
12. Kato, K., Terao, S., Hirata, M., 1988. Studies on scavengers of active oxygen species.1. Synthesis and biological activity of 2-O-alkylascrobic acids. J. Med. Chem. 31,793–798.
13. M.G. Mokganya, M.P. Tshisikhawe., 2019. Medicinal uses of selected wild edible vegetables consumed by Vhavenda of the Vhembe District Municipality, South Africa. South African Journal of Botany 122, 184–188.
14. Maanda, M.Q., Bhat, R.B., 2010. Wild vegetable use by Vhavenda in the Venda region of Limpopo Province, South Africa. Phyton - International Journal of Experimental Botany 79, 189.
15. Maroyi A., 2011. The Gathering and Consumption of Wild Edible Plants in Nhema Communal Area, Midlands Province, Zimbabwe. Ecology of Food and Nutrition 50: 506–525.
16. Molla, M.H.; Ahsan, M.S.; Alam, M.T. and Haque, M.E., 2010. Antibacterial activity in the leaves of seven bitter medicinal plants of Bangladesh. J. Bio. Sci., 18:128-133.
17. Naik V. N., 1998. "Flora of Marathwada" Vol-I and Vol-II. Amrut Prakashan Aurangabad.
18. Nascimento, G.G, Locatelli, J, Freitas, P.C. and Silva, G.L., 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol., 31(4):247-256.
19. Nesamvuni, C., Steyn, N.P., Potgieter, M.J., 2001. Nutritional value of wild, leafy plants consumed by the Vhavenda: Research letter. South African Journal of Science 97,51–54.
20. Obeidat, M.; Shatnawi, M.; Al-Alawi, M.; Al-Zu Bi, E.; Al-Dmoor, H.; Al-Qudah, J.; El-Qudah, M. and Otri, I., 2012. Antimicrobial activity of crude extracts of some plant leaves. Res. J. Microbiol., 7:59-67.
21. R.N. Gacche, N.A. Dhole., 2011. Profile of aldose reductase inhibition, anti-cataract and free radical scavenging activity of selected medicinal plants: An attempt to standardize the 42botanicals for amelioration of diabetes complications, Food and Chemical Toxicology 491806–1813.
22. Rahman, K.M.M., Sousa-Poza, A., 2010. Food consumption and nutritional status of vulnerable people rearing livestock in Bangladesh. Bangladesh Journal of Agricultural Economics XXXIII, 41–56.
23. Rajasree, R.S., Sibi, P.I., Francis, F., William, H., 2016. Phytochemicals of Cucurbitaceae family – A review. International Journal of Pharmacognosy and Phytochemical Research 8, 113–123.
24. Rebecca L. Siegel, Kimberly D. Miller, Hannah E. Fuchs, Ahmedin Jemal., 2021. Cancer Statistics, Cancer J Clin. 2021; 71:7–33.
25. Sekeroglu, N. and Koca U.,2010. Current status of medicinal and aromatic plants production and trade in Turkey. Biomed., 5(2):65-73.

26. Susan J. Curry, Tim Byers, and Maria Hewitt., 2003.Lifestyle Behaviors Contributing to theBurden of Cancer, National Academies Press (US); ISBN: 0-309-50619-0, pages564.
27. Trease GE, Evans WC. Pharmacognosy. 14th Edition, W.B. Scandars Company, Ltd. London, 1989:69 - 300.
28. World Health Organization (WHO). Global Health Estimates 2020: Deaths by Cause, Age, Sex, by Country and by region, 2000-2019.
29. Yu, W., Zhao, Y., Shu, B., 2004. The radical scavenging activities of Radix puerariae isoflavonoids: a chemiluminescence study. Food Chem. 86, 525–529.

Signature

Mr. Sachin S. Shinde
Research Scholar

Signature

Prof. Dr. B. D. Gachande
Research Guide